K102432

JAN 14 2011

510(k) SUMMARY

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92

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Date the Summary was Prepared:	Aug, 25, 2010; revised Jan 5, 2011
Name of the Device	25-OH Vitamin D Assay Kit 25-OH Vitamin D Assay Calibrator Set 25-OH Vitamin D Assay Control Kit
Trade Name:	25-OH Vitamin D Assay Kit 25-OH Vitamin D Assay Calibrator Set 25-OH Vitamin D Assay Control Kit
Common/Usual Name	Vitamin D Assay
Device Classification Name	Vitamin D Test System
Product code:	MRG – Vitamin D Test System JIS – Calibrator, Primary JJX – Single (specified) Analyte Controls (Assayed and Unassayed)
Panel:	Chemistry (75)
Submission Type	510k

Regulation Number	21 CFR 862.1825 – Vitamin D Test System 21 CFR 862.1150 – Calibrator, Primary 21 CFR 862.1660 – Quality Control material (Assayed and Unassayed)
Device Class	II (Assay) II (Calibrator) I (Controls),
Predicate Device:	The 25-OH Vitamin D Assay is substantially equivalent to the currently marketed 25-OH Vitamin D EIA (k021163).
Manufacturing Address	Diazyme Laboratories 12889 Gregg Court Poway, CA 92121 USA
Establishment Registration	2032900

DESCRIPTION OF THE DEVICE

The 25-hydroxy (25-OH) Vitamin D assay is based on the principle of α-complementation of the enzyme β-galactosidase and the competition between an enzyme donor-25-OH Vitamin D conjugate, Vitamin D binding protein and the 25-OH Vitamin D content of a serum sample. Samples with higher 25-OH Vitamin D concentrations produce higher β-galactosidase activities and *vice versa*. Chlorophenol red-β-D- galactopyranoside (CPRG) is used as the enzyme substrate and the accumulation of the reaction's product (chlorophenol red) is monitored at 560 nm. The 25-OH Vitamin D concentration of a patient sample is proportional to the measured β-galactosidase activity. The assay consists of a sample extraction step during which Vitamin D is irreversibly dissociated from its serum transporter. Extracted samples are then analyzed on a microplate reader after the sequential addition of three reagents.

25-OH Vitamin D assay calibrator set is intended for use with the Diazyme 25-OH Vitamin D assay kit only. Six calibration levels are needed for each run. Calibrators are treated exactly the same as patient samples.

25-OH Vitamin D assay 2-point control set is intended for use with the Diazyme 25-OH Vitamin D assay kit only. Controls are treated exactly the same as patient samples. The quality controls assist laboratory users in verification steps ensuring that the assay reagents are functioning correctly. Users are instructed to verify the calibration curve with the controls and run controls each time a new lot of reagents are received.

INDICATIONS FOR USE

The Diazyme 25-OH Vitamin D assay is designed for the quantification of total 25-OH Vitamin D in human serum and plasma. The assay results are to be used in parallel with other clinical data to assess the Vitamin D status of a patient. For *in vitro* diagnostic use only.

The 25-OH Vitamin D assay calibrator set is intended for use in the calibration of the Diazyme 25-OH Vitamin D assay kit only. For *in vitro* diagnostic use only.

The 25-OH Vitamin D assay control kit is intended for use as quality controls for the Diazyme 25-OH Vitamin D assay kit only. For *in vitro* diagnostic use only.

Table 1 Summary of Assay Kit Components

IDS 25-OH Vitamin D EIA (predicate k021163)	**Diazyme 25-OH Vitamin D Assay**
Kit can be only used for the 25-OH Vitamin D quantification on microplate readers.	Kit can be only used for the 25-OH Vitamin D quantification on microplate readers.
Antibody Coated Plate (MICROPLAT) • Microplate with 25-OH Vitamin D sheep polyclonal antibody linked to the inner surface of the polystyrene wells, 12 x 8 well strips in a foil pouch with desicant.	Microplate • Non-coated, polystyrene, round bottom, microplate (96 wells, in a plastic pouch). Microtube rack, 8-well microtube strips.
Adhesive plate scaler • 8 per kit	Adhesive plate sealer • 3 per kit
Dissociation buffer (25-D Biotin) 1 bottle • Proprietary reagent for dissociating Vitamin D • 25-OH Vitamin D labeled with Biotin • Stabilizers	Extraction solution (EX) 1 bottle • Acetonitrile containing solution
Wash Buffer (WASHBUF) 1 bottle • Phosphate buffered saline containing Tween	NA
Enzyme Conjugate (ENZYMCONJ) 1 bottle • Phosphate buffered saline containing avidin linked to horseradish peroxidase, protein, enzyme stabilizers and preservatives.	Reagent 1 2 bottles • R1a (1 bottle): Lyophilized enzyme acceptor, Vitamin D binding protein and phosphate salts. • R1 (1 bottle): Reconstitution buffer containing phosphate salts and preservatives.
TMB substrate (SUBS) 1 bottle	Reagent 2 2 bottles

• Proprietary aqueous formulation of tetramethylbenzidine (TMB) and hydrogen peroxide.	• R2a (1 bottle): Lyophilized enzyme donor. • R2 (1 bottle): Reconstitution buffer containing phosphate salts and preservatives.
NA	Reagent 3 2 bottles • R3a (1 bottle): Lyophilized substrate (CPRG). • R3 (1 bottle): Reconstitution buffer containing phosphate salts and preservatives.
STOP solution (HCl) 1 bottle • Proprietary aqueous formulation of tetramethylbenzidine (TMB) and hydrogen peroxide.	STOP reagent 1 bottle • Concentrated sodium carbonate solution.
Calibrator set	Calibrator set
1 x 1.0 mL Calibrator 0 1 x 1.0 mL Calibrator 1 1 x 1.0 mL Calibrator 2 1 x 1.0 mL Calibrator 3 1 x 1.0 mL Calibrator 4 1 x 1.0 mL Calibrator 5 1 x 1.0 mL Calibrator 6	1 x 1.0 mL Calibrator 1 1 x 1.0 mL Calibrator 2 1 x 1.0 mL Calibrator 3 1 x 1.0 mL Calibrator 4 1 x 1.0 mL Calibrator 5 1 x 1.0 mL Calibrator 6
Control set	Control set
1 x 1.0mL Control 1 1 x 1.0mL Control 2	1 x 1.0mL Control 1 1 x 1.0mL Control 2

PERFORMANCE TESTING SUMMARIES

Precision Study

Acceptance Criteria: Precision: CV ≤ 10%

The precision of the Diazyme 25-OH Vitamin D Microplate Assay is evaluated according to the Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline. Acceptance Criteria: Precision: CV ≤ 10%.

Seven precision levels were used: three serum controls (containing 21.5 ng/mL, 37.0 ng/mL and 60.8 ng/mL) and four serum samples. Two samples correspond to deficient patients (19.0 ng/mL and 19.4 ng/mL), one sample corresponds to a near cut-off concentration (36.4 ng/mL) and one sample corresponds to an optimum level (43.4 ng/mL).

Furthermore, to test the performance of the assay at the limits of the dynamic range, a severely deficient patient (7.8 ng/mL) and a very high sample (101.2 ng/mL) were subjected to the same precision studies.

The mean value (Mean), standard deviation, within-run imprecision and total imprecision are calculated and summarized in the following tables:

	Deficient Control	**Normal Control-1**	**Normal Control-2**	**Deficient Sample#1**	**Deficient Sample#2**	**Near cut-off Sample**	**Optimal Sample**
Data points	40	40	40	40	40	40	40
Mean (ng/mL)	24.4	38.4	61.7	19.6	19.0	33.0	42.9
Within-run SD (ng/mL)	1.0	2.0	4.0	0.9	1.1	1.5	1.6
Within-run CV (%)	4.2	5.3	6.5	4.8	5.7	4.6	3.6
Total SD (ng/mL)	1.9	3.7	5.9	1.8	1.7	2.8	3.7
Total CV (%)	7.9	9.7	9.6	9.4	9.1	8.4	8.6

	Severely Deficient Sample	Very High Sample
Data points	40	40
Mean (ng/mL)	7.8	101.2
Within-run SD (ng/mL)	1.2	6.6
Within-run CV (%)	15.3%	6.5%
Total SD (ng/mL)	1.3	9.8
Total CV (%)	16.7%	9.7%

Linearity/Reportable Range

To establish the linearity of the assay, study design was used based on the CLSI protocol EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: a Statistical Approach: Approved Guideline.*

Eleven levels of linearity were prepared by diluting a serum sample containing 130 ng/mL of 25-OH Vitamin D with a Vitamin D Diluent (Phosphate Buffer Saline supplemented with 9% of Bovine Serum Albumin). Linearity levels were prepared according to Clinical and Laboratory Standards Institute (CLSI) EP6-A. The samples prepared were tested with the Diazyme 25-OH Vitamin D assay, in triplicates. The results were processed using the EP Evaluator Software (Version 8.0) parameterized to an allowable systematic error of 10.8%. The linearity range was found to be 5.9 -120.2 ng/mL.

LoB/LoD/LoQ

The Limit of Blank (LoB), the Limit of Detection (LoD) and the Limit of Quantitation (LoQ) of the Diazyme 25-OH Vitamin D assay on microplate were determined according to CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation. The following are the limits determined with the Diazyme 25-OH Vitamin D assay method:

LoB = 1.0 ng/mL

LoD = 3.6 ng/mL

LoQ = 5.9 ng/mL

Analytical specificity

Interference Study

The Diazyme 25-OH Vitamin D assay was subjected to an interference study according the CLSI EP7-A2 protocol. The following substances normally present in the blood produced less than 10% deviation when tested at levels equal to the concentrations listed below:

Interference	**Concentration**
Ascorbic Acid	10 mM
Free Bilirubin	40 mg/dL
Conjugated Bilirubin	30 mg/dL
Triglyceride	500 mg/dL
Hemoglobin	100 mg/dL

Cross Reactivity

The Diazyme 25-OH Vitamin D assay was tested for cross reactivity to the following Vitamin D metabolites:

Cross reactant	**Concentration tested**	**Cross reactivity**
25-OH Vitamin D3	100 ng/mL	100.0%
25-OH Vitamin D2	100 ng/mL	92.2%
1,25-OH Vitamin D3	100 ng/mL	3.9%
1,25-OH Vitamin D3	100 ng/mL	2.6%
Vitamin D3	100 ng/mL	-0.6%
Vitamin D2	100 ng/mL	2.9%

Comparison Studies

Method Comparison

Human serum samples were tested with the Diazyme 25-OH Vitamin D assay and the obtained results were compared to the predicate method. A total of 80 samples (ranging from 7.7 to 113.8 ng/mL of 25-OH Vitamin D) were tested in both assays. The above described accuracy study showed that the Diazyme 25-OH Vitamin D Microplate Assay results correlated well with predicate method with a correlation coefficient of 0.936 with a slope of 1.039 and -2.469 intercept.

	Serum Samples
n	79
Slope	1.039
Intercept	-2.481
Correlation coeffi-	0.935
Range of values	7.7 ng/mL- 113.8 ng/mL

Matrix Comparison

26 samples were used in a comparison study between serum and Li-Heparin plasma. Linear regression of the "Serum versus Li-Heparin plasma" data yielded the following results: y = 0.992x-0.173 and R^2 = 0.986.
55 samples were used in a comparison study between serum and K3-EDTA plasma. Linear regression of the "Serum versus K3-EDTA plasma" data yielded the following results: y = 0.908x + 1.749 and R^2 = 0.981.

Reference Range Study

To determine a reference range for the Diazyme 25-OH Vitamin D microplate assay, the 25-OH Vitamin D serum concentrations of a US population of 150 apparently healthy individuals were measured with the Diazyme method. Thirty (30) samples from Pennsylvania (Northern U.S.) were collected from an FDA Licensed Donor Center with informed consent through Dx Biosamples. Sixty (60) samples from Tennessee (Central U.S.) and sixty (60) samples from Texas (Southern U.S.) were collected according to an IRB approved protocol by ProMedDx, LLC.

All participating individuals met the following inclusion conditions:

- The age of all individuals was within the 21-90 years old range.
- Individuals were from three different geographical locations: 30 from Pennsylvania (Northern US), 60 from Tennessee (Central US) and 60 from Texas (Southern US).
- All samples were collected during the months of October and November 2010 (fall season).
- The studied population consisted of 63 light skin individuals (42%) and 87 dark skin individuals (58%).

- 148 individuals (98.6%) did not take any artificial Vitamin D supplements. 2 individuals (1.4%) did take some Vitamin D supplements but did not exceed the dose of 2000 IU.
- All 150 individuals did not have any family history of parathyroid or calcium regulatory disease.
- All 150 individuals did not have any history of kidney disease, GI disease, liver disease, calcium-levels related disease, thyroid disease, parathyroid disease, calcium related disease, seizures, chronic disease or bariatric surgery.
- All 150 individuals were not currently taking any medications that are known to affect absorption or catabolism of Vitamin D (including cholesterol absorption inhibitors such as Vytorin®, Inegy™ or Zetia; anticonvulsants such as Neurontin, Depakine® and Trileptal; glucocorticoids such as Cortisol, Prednisone and Dexamethasone; HAART (AIDS treatment) or antirejection medications.

Analysis of the reference range study data yielded the following results:

- Lowest 25-OH Vitamin D concentration: 9.9 ng/mL.
- Highest 25-OH Vitamin D concentration: 65.8 ng/mL.
- Median 25-OH Vitamin D concentration: 21.1 ng/mL
- Observed range (2.5th to 97.5th percentile): 11.3 to 41.4 ng/mL.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Diazyme Laboratories, Inc.
c/o Abhijit Datta
Quality Assurance Specialist
12889 Gregg Court
Poway, CA 92064

JAN 14 2011

Re: k102432
Trade Name: Diazyme 25-Hydroxy Vitamin D Microplate Assay Kit, Diazyme 25-Hydroxy Vitamin D Assay Calibrator Set, Diazyme 25-Hydroxy Vitamin D Assay Control Kit
Regulation Number: 21 CFR §862.1825
Regulation Name: Vitamin D Test System
Regulatory Class: Class II
Product Codes: MRG, JIS, JJX
Dated: January 5, 2011
Received: January 6, 2011

Dear Abhijit Datta:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Courtney Harper, Ph.D.
Director
Division of Chemistry and Toxicology
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number k102432:

Device Name: Diazyme 25-hydroxy Vitamin D Assay Kit

Indications for Use:

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The 25-hydroxy Vitamin D Assay Control kit is intended for use as quality controls for the Diazyme 25-OH Vitamin D Assay kit only. For *in vitro* diagnostic use only.

Prescription Use X AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

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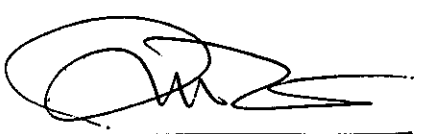
Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)



Division Sign-Off

Office of In Vitro Diagnostic
Device Evaluation and Safety

510(k) K102432